

#### RESEARCH ARTICLE

# Impact of maintenance regimes on species richness in newly established perennial wildflower meadows

#### Maria Hristova

University of Forestry, Sofia

Corresponding author: Maria Hristova (m.pl.hristova@gmail.com)

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#### **Abstract**

Perennial wildflower meadows contribute to the enhancement of the quality of urban green spaces, offering benefits for both people and wildlife. Significant impact is attributed to management in fostering species-diverse plant communities. However, there are not enough studies exploring the influence of early maintenance regimes on plant diversity. A three-year experiment was conducted to investigate the impact of maintenance regimes on newly established perennial wildflower meadows and the influence of sowing mix on species richness. Three types of perennial wildflower seed mixes were sown on arable land and managed differently during the initial year. Results indicate statistical significance for each main effect, including maintenance type and sowing mix, with no significant interaction between these factors. Mowing during the early growth stage is shown to increase species richness, while no significant difference is observed between weeded and unmanaged plots. The dry meadow sowing mix demonstrates the highest diversity index (Shannon-Weaver, 1963). Maintenance regimes exhibit a significant influence on species richness independently of the sowing mix used. Furthermore, mowing during the early growth stage is found to enhance species diversity in the long term. These findings provide beneficial insights for the more efficient management and establishment of species-rich wildflower perennial meadows in both urban and suburban environments, particularly in areas with a significant soil weed seed bank.

#### **Keywords**

biodiversity, urban meadows, wild flowers, maintenance, mowing

#### Introduction

Urban development increasingly disturbs natural habitats. This is the reason for creating more diverse urban green spaces. Flowering meadows provide a rich diversity

of perennials, both forbs and grasses. They have a positive impact on citizens with their ornamental effect (Wood et al., 2018). Using native perennial seed mixes improves the quality of urban greenery (Norton et al., 2019) and conserves biodiversity (Bretzel et al., 2016). An essential requirement for using native seeds is that they originate from the same biogeographic zone (Mainz & Wieden, 2019). There is evidence for the positive effect of a lower sowing rate on species richness, especially when seeds are sown in unproductive soils, weed-free mulches, or when the initial fast effect of rapid forb development is not required (Jiang & Hitchmough, 2022).

Maintenance plays a significant role in preserving species richness. Mowing frequency is an important factor for the number of species in the meadows. Decreasing mowing times to twice per growing season in existing grass areas increases the number of plant species in the long term (Chollet et al., 2018; Sehrt et al., 2020; Wastian et al., 2016). There is one more advantage to the variety of plant species: Species richness is associated with flower diversity and abundance, which provide food for more wild species like pollinator insects (Lindemann-Matthies and Bose, 2007; Wastian et al., 2016). There is an opportunity for providing habitat and food for wildlife animals by leaving unmown parts or by gradually mowing some parts, leaving others (Johansen et al., 2019).

The mowing regime manages the flowering period and duration. The appropriate mowing regime increases flowering. Establishing optimal mowing frequencies throughout the year influences the preservation of dicotyledon diversity. This is particularly important in fertile soils, as they create a competitive environment that favors the growth of monocotyledonous grass species, especially in phosphorus-rich soils (Piqueray et al., 2019). Mowing in combination with collecting hay decreases nitrogen and iron in the soil and plays a key role in increasing plant diversity (Chollet et al., 2018; Manninen et al., 2010). Mowing is a significant factor in maintaining species quantity in the long term.

There are seven significant factors related to future meadow establishments based on stakeholder manager perceptions: "aesthetics and public reaction, spatial context, human resources and economic sustainability, local politics, communication, biodiversity and habitat value, and physical factors affecting establishment and maintenance" (Hoyle et al., 2017). The first three factors are described as the most influential. Cities could introduce perennial meadows to boost biodiversity and enhance the aesthetic appeal of urban green spaces. There is a challenge for local authorities from an economic point of view, such as removing and composting of meadow arising. There is a need of more detailed economic research on cutting frequency and species that produce less biomass. Frequent cutting could be cheaper because it involves dispersing the removal over the site, in contrast with cutting once a year, which requires collecting and composting the removed biomass. Using shorter plant species or species from unproductive soil habitats could decrease plant biomass and the cost of disposal. From this perspective, selecting the right seed mix is crucial. It is appropriate to use plants from less productive habitats, which produce less biomass.

There is much evidence for the positive effect of mowing to preserve and increase species richness, but there is not enough research on different types of maintenance in initial plant growth to create species-rich flowering meadows. The aim of this research is to examine the impact of various maintenance practices on the initial growth of meadows, utilizing three distinct regimes and three different perennial wildflower mixes, and to assess their effects on species richness.

## Material and methods

# **Experiment establishment and field studies**

In Bulgaria, there are no native perennial seed mix producers. This was the reason for using wildflower species from Hilzingen, Germany, which is in the same biogeographic zone as Sofia, Bulgaria. (Europe's Biodiversity - Biogeographical Regions and Seas — European Environment Agency, 2002). Flowering meadows were established using three distinct types of wildflower perennial seed mixes, each sourced from nursery-raised wildflower plants. These mixes include: Mix 02 - Wet meadow seed mix, Mix 06 - Universal meadow seed mix, and Mix 06a - Dry meadow seed mix. Table 1. presents the plant species contained within various seed mixes. With the exception of Buphthalmum salicifolium L. and Rhinanthus alectorolophus (Scop.) Pollich, all species are native to the Bulgarian flora. The plots are situated in the Vrazhdebna Training and Experimental Field Centre, Sofia, Bulgaria. The soil in the area is alluvial-meadow, slightly gravelly. The latest soil analysis data from Megatron (2022) shows an average level of organic matter (3-6%), a low level of ammonium  $(NH_4-N)$ =02-04kg/ha) and nitrogen ( $NO_3$ -N = 10-30kg/ha). The soil has high quantities of phosphorus (P > 45 mg/kg (ppm)), low quantities of potassium (K = 60-120 mg/kg(ppm)), and neutral acidity (pH = 6.3-7.0). The soil structure is mostly sandy (40– 60%) with low quantities of loam (10-20%) and average silty content (20-40%). Plant available water value is low (50-65% of field capacity).

The climate is European continental. The average annual precipitation is 650 mm and 380 mm of it is during the growing season. The driest months are December, January, and February; with the highest precipitations being May, June, and July (Koleva, 1990).

The soil preparation includes applying a rotary tiller to the top soil and removing all plant parts, roots, and others; levelling with a rake; and removing large parts from the surface. Every seed mix has four samples. Each of them is 10 m<sup>2</sup>. Seeds were spread in the middle of April 2021, raked shallow (1-2 cm), and compacted with a plank. During the germination period, they were irrigated in extremely dry periods with quantities that enrich the top 2 cm of soil with an approximate amount of water. Three types of maintenance were applied at the end of May in the first year: weeding, mowing, and control, which were left without any interventions. There are two mowed plots, one weeded and one control, for every seed mix.

**Table 1.** Plant species in seed mixes

No	C	Seed mixes		
) <b>N</b> o	Species		06	06a
1	Achillea millefolium L.	+	+	+
2	Agrimonia eupatoria L.		+	
3	Anthemis tinctoria L.		+	+
4	Anthoxanthum odoratum L.		+	+
5	Anthriscus sylvestris (Hoffm.) Link	+		
6	Anthyllis vulneraria L.		+	+
7	Arrhenatherum elatius (L.) P.Beauv.	+		
8	Bellis perennis L.	+		
9	Briza media L.			+
10	Bromus erectus Huds.			+
11	Buphthalmum salicifolium L.		+	+
12	Campanula patula L.	+	+	+
13	Campanula rotundifolia L.			+
14	Carum carvi L.		+	
15	Centaurea jacea L.	+	+	+
16	Centaurea scabiosa L.		+	+
17	Cichorium intybus L.		+	
18	Crepis biennis L.	+	+	
19	Cynosurus cristatus L.	+	+	+
20	Daucus carota L.	+	+	+
21	Dianthus carthusianorum L.		+	+
22	Echium vulgare L.		+	
23	Festuca ovina L.			+
24	Festuca rubra var. trichophylla (Vill.) Gaudin			+
25	Galium mollugo L.	+	+	
26	Galium verum L.		+	+
27	Geranium pratense L.		+	
28	Heracleum sphondylium L.	+		

Nº	Species	Seed mixes		
JNō		02	06	06a
29	Hypericum perforatum L.		+	+
30	Knautia arvensis (L.) Coulter	+	+	+
31	Leontodon hispidus L.	+		+
32	Leucanthemum vulgare (Vaill.) Lam.	+	+	+
33	Lotus corniculatus L.		+	+
34	Malva moschata L.		+	+
35	Medicago lupulina L.		+	+
36	Onobrychis viciifolia Scop.	+	+	+
37	Picris hieracioides L.	+		
38	Pimpinella saxifraga L.		+	+
39	Plantago lanceolata L.	+	+	
40	Potentilla recta L.		+	
41	Prunella grandiflora (L.) Scholler	+	+	+
42	Prunella vulgaris L.	+	+	
43	Ranunculus acris L.	+		
44	Ranunculus bulbosus L.	+		+
45	Rhinanthus alectorolophus (Scop.) Pollich		+	+
46	Rumex acetosa L.	+		
47	Salvia pratensis L.	+	+	+
48	Salvia verticillata L.		+	
49	Sanguisorba minor Scop.	+	+	+
50	Scabiosa columbaria L.			+
51	Silene nutans L.		+	
52	Silene vulgaris (Moench) Garcke	+	+	+
53	Thymus pulegioides L.			+
54	Tragopogon orientalis L.		+	+
55	Trisetum flavescens (L.) Beauv.	+	+	
56	Veronica teucrium Crantz			+

Legend: 02 – Wet meadow seed mix, 06 – Universal meadow seed mix, 06a – Dry meadow seed mix

On 27 Jul. 2021, all experimental plots were cut down. After that, they were irrigated two times per week in dry weather until the middle of September 2021. The annual weeds were removed in November 2021. In the second year, all plots were cut down on 8 Aug. 2022, and on 27 Jul. 2023.

Data collection took place in the third year (2023), where information regarding the percentage range of species cover abundance and the Braun-Blanquet scale (Barkman et al., 1986; van der Maarel, 1979) was recorded two times in June. Each sample area of 10 m<sup>2</sup> was evaluated and reported accordingly. Number of plant species in each sample were recorded twice (in May and June) or once (in July and September) per month from May to September 2023. The plant species were identified according Delipavlov (2011), Yordanov (1963) and Fetovadzhieva (1973). Non-native for Bulgaria species were identified according to seed mix specifications.

## Data analysis

The dependent variable, species richness, was determined by the total number of observed plant species recorded between May and September 2023. Two independent variables were considered: maintenance regime and seed mix. To assess the impact of initial maintenance type and seed mixes on species richness, a two-way analysis of variance (ANOVA) was performed using SPSS Statistics 28.0.0. A comparison between various maintenance regimes and seed mixes was conducted using the Tukey's Honestly Significant Difference (HSD) test.

The species diversity index H' (Shannon-Weaver, 1963) and index of species evenness E (Pielou, 1966) were calculated for each sample based on species abundance.

**Formula (1)** for determining the species diversity index (H`) (Shannon-Weaver, 1963):

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H' = -\Sigma(C).ln(C)
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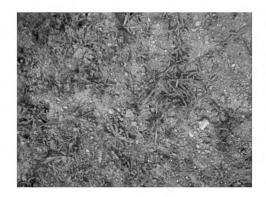
Where: C = the relative abundance of the species.

**Formula (2)** for determining index of species evenness (E) (Pielou, 1966): E = H'/ln(S)

Where: H' = Shannon-Weaver index; S = number of species in the sample.

## Results

Rapid weed emergence commenced in mid-May during the first growing season. Weeds such as: Amaranthus retroflexus, Agropyron repens, Alopecurus myosuroides, Anthemis arvensis, Capsella bursa-pastoris, Chenopodium album, Convolvulus arvensis, Cynodon dactylon, Galinsoga parviflora, Lithospermum arvense, Lolium temulentum, Panicum sanguinale, Plantago major, Polygonum lapathifolium, and Setaria viridis. The coverage of each plot reached 80% at the end of May, when the first maintenance had been applied (Figure 1.). The majority of the plants were Amaranthus retroflexus, Polygonum lapathifolium, and Panicum sanguinale.



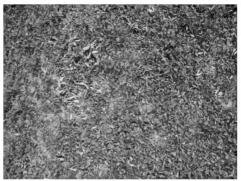




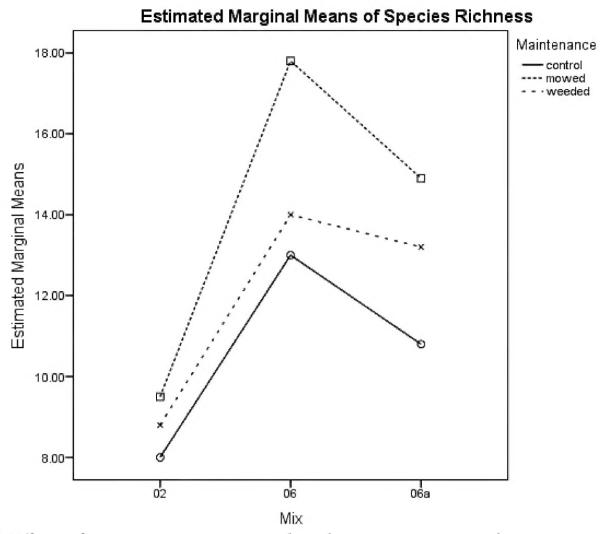
Figure 1. Different types of maintenance regimes in the first year: weeded (left), mowed (middle), control (right)

**Table 2.** The means and standard deviations of species richness under different maintenance regimes.

Seed mix**	Maintenance*	M	SD
	Control	8,00	1,41
Wet meadow	Mowed	9,50	1,84
	Weeded	8,80	1,92
	Control	13,00	5,43
Universal meadow	Mowed	17,80	6,86
	Weeded	14,00	4,85
	Control	10,80	3,27
Dry meadow	Mowed	14,90	3,11
	Weeded	13,20	2,49

**Legend:** 02 – Wet meadow seed mix, 06 – Universal meadow seed mix, 06a – Dry meadow seed mix

After the interventions, the vegetative cover started to recover. The rapidest growth was observed in the mown plots, and on the 10th day, the cover had reached 55-70%. In weeded plots, vegetation appeared more slowly and reached coverage between 10-15%. In control plots, coverage was between 85 and 90%. The first detected species from the sown seed mixes were Bellis perennis, Silene vulgaris and Daucus carota. After mowing all the plots at the end of July, annual weeds had disappeared and the rosettes of seeded perennial plants had shown. After the irrigation started, Setaria viridis had become the most common species. At the end of the first growing season, Dianthus carthusianorum, Tragopogon orientalis, Centaurea jacea, and Leontodon autumnalis were blooming. There were rosettes of Echium vulgare, Anthyllis vulneraria, Malva moschata, Achillea millefolium, Plantago lanceolata, Leucanthemum vulgare, and Knautia arvensis. Campanula patula appeared in the second year but disappeared thereafter. Most of the seeded plant species were observed. There were no individuals in the experimental plots from Pimpinella saxifraga, Anthoxanthum odoratum, Carum carvi, Agrimonia eupatoria, Anthriscus sylvestris, Campanula rotundifolia, Geranium pratense, Heracleum sphondylium, or Rhinanthus alectorolophus. Some native species, like Viscaria vulgaris, Lychnis flos-cuculi, Centaurea cyanus, Holcus lanatus, Trifolium incarnatum, and Coronilla varia, were noticed in the third year.



**Figure 2.** Effects of maintenance regimes and seed mixes on species richness

Silene nutans, Veronica teucrium, Lotus corniculatus, Anthemis tinctoria, Ranunculus acris, Potentilla recta, and Trisetum flavescens were self-seeded in other plots. Briza media was found only in the weeded plot of the dry meadow mix, and Malva mos*chata* was found in the mown plot of the universal meadow mix.

# Maintenance regime

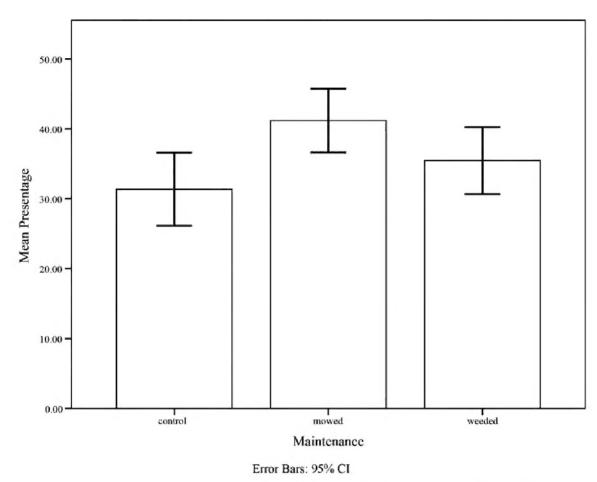
The results of the two-way analysis of variance (ANOVA) for the number of germinated species under different maintenance regimes are presented in Table 2. There is a significant main effect for maintenance, F(2, 51) = 3.916, p = 0.026; a significant main effect for seed mixes, F(2, 51) = 10.80, p < 0.001; and no significant interaction between maintenance type and seed mix, F(4, 51) = 0.446, p = 0.774.

On Figure 2. it is presented that there is no interaction between the main effects. Estimated marginal means are highest in weed plots and lowest in control plots.

A post hoc comparison using Tukey's HSD test indicated that the mean score for mowed plots (M = 14.07, SD = 5.56) was significantly different (p = 0.009) than the controls (M = 10.60, SD = 4.07). However, the weeded (M = 12.00, SD = 3.89) did not significantly differ from the mowed and controls. Taken together, these results suggest that early mowing in the first year does have a positive effect on species richness. Weeding does not appear to be a significant maintenance factor for species richness.

Figure 3. below shows that the mowed plots have more established sown species in comparison with others.

The Shannon-Weaver diversity index (Shannon-Weaver, 1963) (Figure 4.) shows that mown plots have the highest diversity in comparison with weeded and control plots. However, mown plots have the lowest index of evenness (Pielou, 1966), in contrast with weeded and control plots, which are equal. It means that some species in mown plots had started to dominate.



**Figure 3.** The mean percentage ratio between the established species from the seed mixes and the total number of species for all seed mixes under various maintenance regimes.

### Seed mix

A post hoc comparison using the Tukey's HSD test for seed mixes indicated that the mean score for the wet meadow mix 02 (M = 8.95, SD = 1.79) was significantly different (p<0.05) than the universal meadow mix 06 (M = 15.65, SD = 6.02) and dry meadow mix 06a (M = 13.45, SD = 3.33). The universal meadow mix 06 (M = 15.65, SD = 6.02) did not significantly differ from the dry meadow mix 06a (M = 13.45, SD = 3.33). Consequentially, there is no significant difference between dry meadow and universal seed mix species richness.

The species diversity index (H`) (Shannon-Weaver, 1963) (Figure 5.) indicate that the plots with the highest index of diversity are those with a dry meadow seed mix. The plots with the highest index of species evenness (E) (Pielou, 1966) are those with a wet meadow seed mix. The plots with the lowest diversity and evenness index are those with a universal meadow mix.

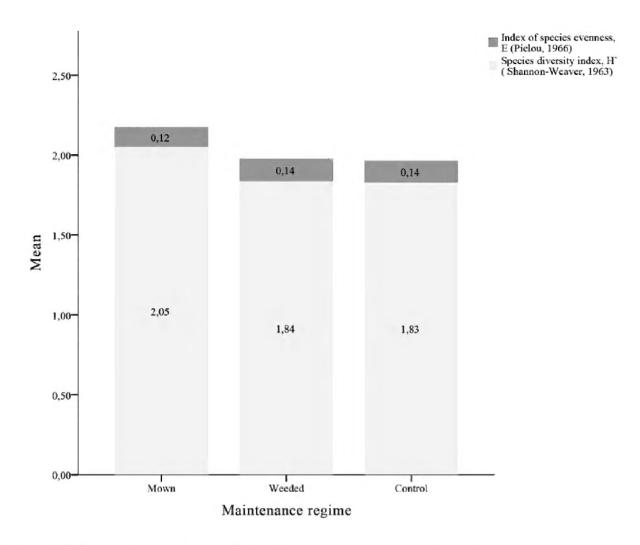


Figure 4. Alpha diversity for different maintenance regimes (species diversity index H' (Shannon-Weaver, 1963), index of species evenness E (Pielou, 1966))

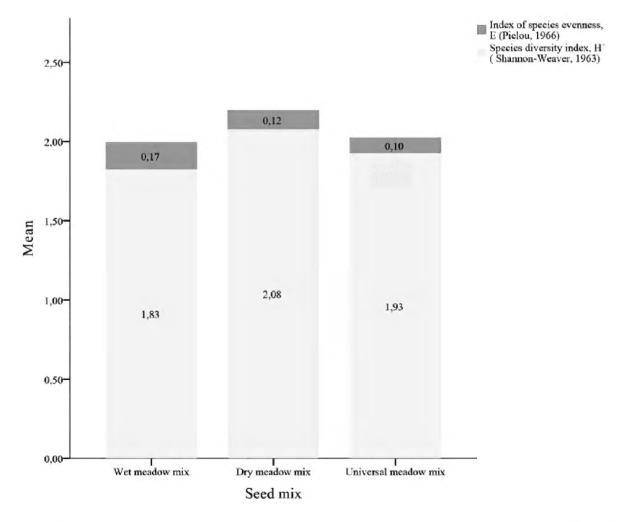


Figure 5. Alpha diversity for different seed mixes (species diversity index H' (Shannon-Weaver, 1963), index of species evenness E (Pielou, 1966))



**Figure 6.** The beginning of the flowering season in the third year (2023)



**Figure 7.** Blooming in the middle of May 2023 (third year). Mix 02 (left), mix 06 (middle), mix 06a (right)

Figures 6. and 7. show flowering at the beginning of the third vegetative season. There are Tragopogon orientalis, Onobrichis viciifolia, Leucanthemum vulgare, Salvia pratensis, and Dianthus carthusianorum blooming.

# **Discussion**

The maintenance regime in the first year is a significant factor for species richness in the establishment and development of newly established wildflower meadows in the long term. Early mowing has a positive effect on removing annual weeds. The cutting allows more light to enter the soil surface for wildflower species germination. Mowing increases the diversity index (Shannon-Weaver, 1963) but leads to lower evenness index (Pielou, 1966). Some species are not adapted to mowing or require more frequent mowing to decrease dominant species abundance. A further research about this effect is needed.

Weeding has no significant impact. In some cases, weeding could lead to less diverse communities because of a human factor. Some plants from the sown seeds might be inadvertently removed during the weeding process. The other reason for the lower diversity of weeding plots might be the microclimate. However, there were species with individuals only in mowed or weeded plots. The microclimate of mowed communities differs from that of weeded communities. Weeds create partial shade on the ground. Removing them allows direct sunlight to reach the ground, increasing the amount of sunlight on the soil surface. However, this also leads to a rise in temperature and faster water evaporation. Shading the ground retains moisture in the soil for longer and provides better conditions for germination (Bringmann et al., 2006).

Mowed and control plots have a significant difference in plant species richness. Leaving plots without any intervention in the first months is the worst scenario for species richness. Weeds suppress the germination of perennial plants. However, annual weeds are pioneer species, and their quantity decreases with time at the expense of perennial flowers (Kirmer, 2014). Like pioneer species, weeds play the role of plant community edificators and may provide more suitable conditions for perennial seed germination. Though, if they completely cover the surface, they become a barrier that prevents light from reaching the ground.

As previous findings these results support that cuttings and removing the aboveground biomass once or twice per year increase species richness (see Chollet et al., 2018; Piqueray et al., 2019; Sehrt et al., 2020; Wastian et al., 2016). However, mowing and leaving the aboveground biomass could lead to poor diversity in contrast with setting-aside plots (Hyvönen et al., 2021).

In the seed mix specifications, there was no information about the proportion of seeds from different species. Additionally, it was noted that certain species, such as Bellis perennis and Stachys recta, were found in the plots despite not being included in the seed mix. This was particularly evident in the dry meadow mix.

Future studies could consider conducting experiments using wild regional seeds, examining their field seed germination rates, and evaluating their proportion in the seed mix. Additionally, it would be interesting to explore the reasons for the lower evenness index (Pielou, 1966) observed in mown plots.

This research could benefit urban and suburban greening projects devoted biodiversity enrichment by creating perennial wildflower meadows and to improve the quality and aesthetic of urban environments. The findings would be helpful in the establishment of perennial wildflower seeds, especially in soils with a significant weed seed bank.

# **Conclusions**

Early moving proves to be a successful method for weed suppression during the initial phase of flowering meadow establishment, especially in phosphorus-rich soils. Implementing a mowing regime with two cuttings for the first year, followed by annual cutting thereafter, provides advantage to many perennial species and results in greater plant species diversity in flower meadows. Weeding is not appropriate in the initial growing stage. Species richness is influenced by the maintenance regime, irrespective of the type of seed mix used. Nevertheless, sowing seed mixes designed for dry habitats creates more diverse flowering meadows.

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